Novel *DOCK7* mutations in a Chinese patient with early infantile epileptic encephalopathy 23

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To the Editor: Early infantile epileptic encephalopathy 23 (EIEE23; OMIM #615859) is a rare (<1/1,000,000 worldwide) kind of inherited autosomal recessive disorder. Patients with EIEE23 are characterized by intractable seizures between 2 and 6 months of age, multifocal epileptic activity on electroencephalography (EEG), psychomotor development delay, and cortical blindness or visual impairment.[1] In EIEE23 cases, hypsarrhythmia and abnormalities in speech and brain structure, as well as facial dysmorphism occur.[1] Dedicator of cytokinesis 7 (*DOCK7*) gene, which is a guanine nucleotide exchange factor (GEF) playing a role in axon formation and neuronal polarization, has been identified as a premier genetic etiology for EIEE23.[1] Until now, the spectrum of mutations associated with *DOCK7* that could clarify its causative role in EIEE23 is not fully understood. Moreover, due to the high genetic heterogeneity and rarity of EIEE23, further investigations of novel and specific mutations as well as their associated clinical phenotypes are of great importance.

We report a case of a Chinese family with one affected girl and unaffected parents. The study family was recruited on their first visit at the First People's Hospital of Yunnan Province (Affiliated Hospital of Kunming University of Science and Technology, Kunming, China) on July 11, 2016. This study was approved by the Ethics Committee of the participating institutions and informed consent was signed and obtained from the participants. The affected girl was 3 years old at the time of recruitment, and she is the first child to the healthy non-consanguineous Chinese parents. Besides the prescription of progesterone for preventing miscarriage during early gestation, the pregnancy leading to the normal birth of the candidate was uneventful. Additionally, the girl was born at full term with a birth weight of 2800 g (Chinese normal birth weight: 2500–4000 g). Her neonatal course was also uneventful, except for the diagnosis of atrial septal defect.

She began to present head nodding and infantile spasm about 20 times a day at 6 months of age. She also showed delayed psychomotor development and hypotonia. She could sit, crawl, and stand independently but could not walk stably. She could not speak and had difficulties in daily life. Although the hearing test was normal, she showed no reaction to her name. At 20 months, EGG showed a pattern of hypsarrhythmia, multifocal sharp and spike waves, especially in parietotemperal cortex. Brain MRI performed at the age of 3 years showed abnormally marked pontobulbar sulcus [Figure 1A], mild pontine hypoplasia [Figure 1B], thin corpus callosum and dilation of lateral ventricles [Figure 1C], as well as pachygyria [Figure 1D].

Her parents noticed the lack of ocular contact and reaction to visual stimulus in this girl a few months after her birth. Her ophthalmological examinations showed binocular vision disorders, horizontal nystagmus and left strabismus, the flash evoked visual potentials (FEVP) show abnormal waveform including a longer latency in right eye and decreased amplitude in both eyes, which lead to the diagnosis of cortical blindness. At the age of 3, her occipitofrontal circumference was 47.5 cm (30th percentile). She also showed dysmorphic facial features including low posterior hairlines, protruding ears, highly arched palate, low ear set, gingival maldevelopment, and periorbital fullness.

Metabolic examinations including plasma amino acid and urine organic acid chromatography were normal in this girl. Her previous karyotyping at a resolution of 400 bands

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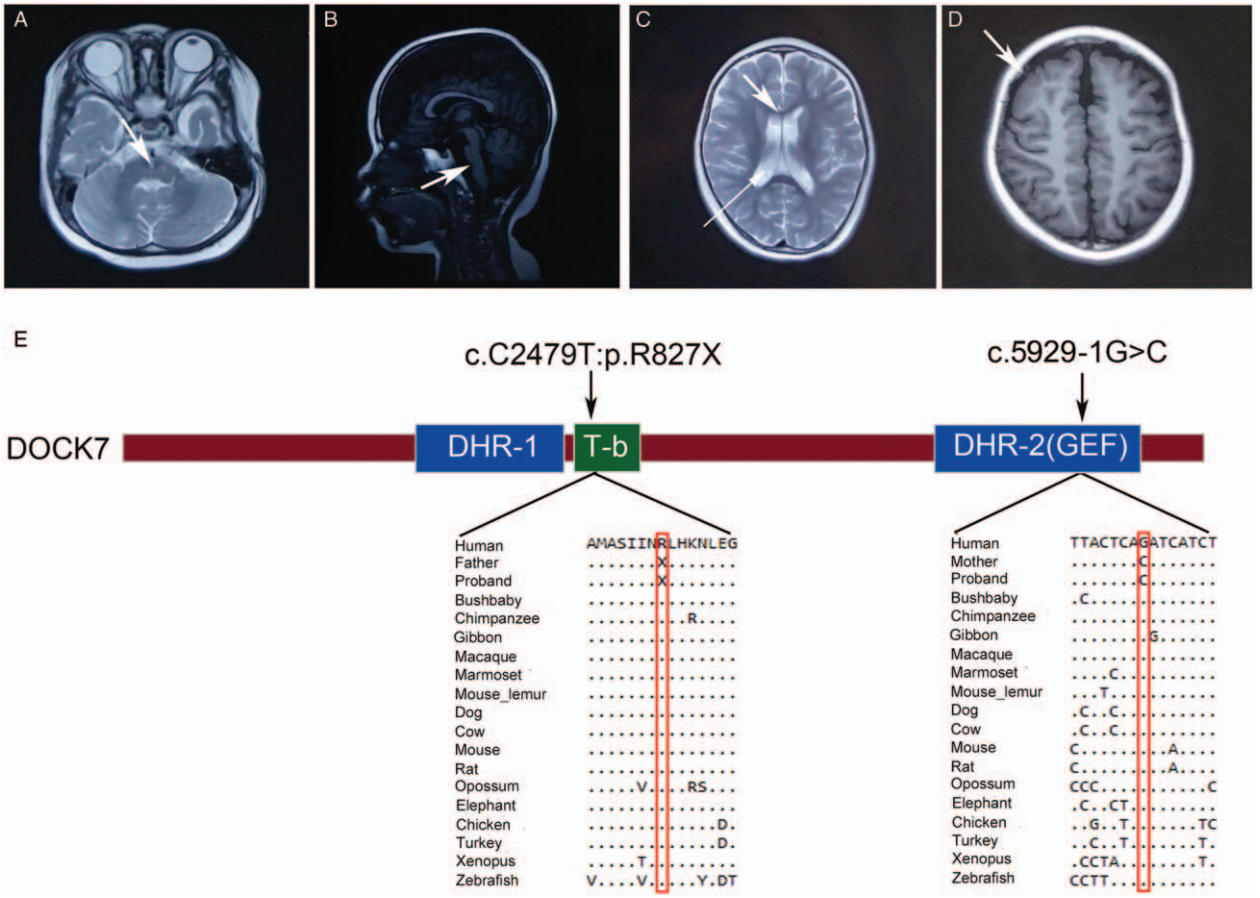

Figure 1: Brain MRI of the patient with *DOCK7* mutations. Locations of abnormality are signified by white arrows. (A) Pontobulbar sulcus. (B) Mild pontine hypoplasia. (C) Dilation of lateral ventricles (long arrow) and thin corpus callosum (short arrow). (D) Pachygyria. (E) A schematic representation of *DOCK7* gene showing DHR-1, DHR-2, and the TACC3-binding region (1, 3), as well as an evolutionary conservation analysis of the two mutations in 17 vertebrates.

and array comparative genomic hybridization (CytoScan; Affimetrix Cytogenetics Whole-Genome 2.7 M Array) were also showed no abnormality.

In July 2016, we sequenced exomes of the affected girl and both her health parents. Whole blood samples were obtained from the family at the First People's Hospital of Yunnan Province. Genomic DNA was extracted using E.Z.N.A.® Blood DNA Kit (cat. no. D3392-02; Omega Bio-tek, Inc, USA). Exomes were captured by the Agilent Sure Select Human All Exon V6 system and sequenced on an Illumina HiSeq2000 platform (Illumina, San Diego, CA, USA). The refined dataset was used to call a high-quality variant set through GATK UnifiedGenotyper.[2] We identified a pair of compound heterozygous mutations in the *DOCK7* gene (NM_001271999;c.5929-1G>C, NM_001271999; c.C2479T; p.R827X; UCSC Genome Browser hg19 assembly) to be the most likely causative variants, which were confirmed by Sanger sequencing.

The DHR1 domain, the catalytic DHR2 domain, and the TACC3 protein-interacting region (T-b region) are necessary for *DOCK7* functionality in cortical neurogenesis, axon development, and neuronal polarity.[3-5] As shown in Figure 1E, variant c.C2479T is located in exon 21 (NM_001271999) and results in a premature stop codon in the anterior part of the T-b region (amino acids 506–1164).[3] The other variant c.5929-1G>C changes the first 5′ splice donor site (G) of exon 46 into C in the DHR2 domain (*DOCK7* UniProt prediction amino acids Q96N67. 1678–2114). It is highly likely that these variants will lead to the premature decay of the corresponding mRNA, affect *DOCK7*'s interaction with TACC3, and disable its DHR2 domain. We further showed through evolutionary conservation analyses that these 2 variant sites are highly conserved among 17 vertebrates [Figure 1E]. Both variants are loss-of-function (LOF) mutations and have never been reported in any public human genetic variant databases such as the 1000 Genomes Project, NHLBI/NIH Exome Sequencing Project, ExAC/gnomAD, ClinVar, or dbSNP.

EIEE23 is a rare and extensively incapacitating disease with an early onset during infancy. Only 3 cases of this disease have been reported to date. Table 1 compared the mutations and clinical symptoms of our study subject with three patients reported by Perrault *et al*.[1] Clinical presentations in our study subject closely matched most of the previously reported cases of EIEE23. For instance, our study subject showed intractable seizures from 6 months and hypsarrhythmia in infancy. Her MRI revealed mild pontine hypoplasia, abnormally marked pontobulbar sulcus and thin corpus callosum. She also suffers from delayed psychomotor development and

Table 1: The matutions and clinical features of our study subject and previously reported cases.

		Perrault *et al* (2014)[1]		
Characteristics	**Our patient**	**Patient A-1**	**Patient A-2**	**Patient B-1**
Age	3	7	5	10
Gender	Female	Female	Female	Female
Mutations	*DOCK7*:c.5929-1G>C Chr1:62941023	*DOCK7*:c.3709C>T (p.Arg1237X) Chr1:62995020	*DOCK7*:c.3709C>T (p.Arg1237X) Chr1:62995020	*DOCK7*:c.983C>G (p.Ser328X) Chr1:63100496
	DOCK7: c.C2479T (p.R827X) Chr1:63021613	*DOCK7*:c.2510delA (p.Asp837Alafs*48) Chr1: 63021582	*DOCK7*:c.2510delA (p.Asp837Alafs*48) Chr1: 63021582	*DOCK7*:c.6232G>T (p.Glu2078X) Chr1:62923324
Age and frequency of seizures attack	6 months, 20 times/day	Between 2 and 4 months	Between 2 and 4 months, 50 times/day	6 months
Types of seizures	Infantile spasms.	Infantile spasms.	Myoclonus, partial complex seizures, tonic seizures.	Eye revulsion, rhythmic arm and body movements, repeated tonic-clonic seizures.
EGG	Hypsarrhythmia, multifocal epileptic activity.	Hypsarrhythmia, multifocal epileptic activity.	Multifocal epileptic activity.	Multifocal epileptic activity.
MRI	Abnormally marked pontobulbar sulcus, mild pontine hypoplasia, thin corpus callosum, dilation of lateral ventricles, pachygyria.	Abnormally marked pontobulbar sulcus, mild pontine hypoplasia, thin and short corpus callosum, atrophy in occipital white and gray matter.		Abnormally marked pontobulbar sulcus, mild pontine hypoplasia, atrophy in the occipital white and gray matter.
Facial features	Low posterior hairlines, highly arched palate, gingivalmaldevelopment protruding ears, low ear set, periorbital fullness, abnormally shaped ears, broad nasal tip, large nasal root.	Low anterior hairline, some periorbital fullness, telecanthus, a broad nasal tip with anteverted nares.	Low anterior hairline, some periorbital fullness, telecanthus, a broad nasal tip with anteverted nares.	Bitemporal narrowness, a low anterior hairline, thick eyebrows, synophrisis, long eyelashes, enophthalmia, large and prominent nasal root, a bulbous nasal tip, a thick and hammered helix, thick earlobes, a short philtrum, full lips and everted lower lip, spaced incisors.
Eye abnormality	Lack of ocular reaction to visual stimulus, binoculus optometric obstacles, horizontal optokinetic nystagmus, left strabismus, cortical blindness.	Lack of ocular reaction to visual stimulus, binoculus optometric obstacles, cortical blindness.	Lack of ocular reaction to visual stimulus, binoculus optometric obstacles, cortical blindness.	Lack of ocular reaction to visual stimulus, cortical blindness, wandering eye movements.
Heart	Atrial septal defect	Aortic supravalvular stenosis, bicuspid valve.		
Language	Lack of speech	Lack of speech	Speak few words	Repeating three words
Pyschomotor development	Walking unstably at 28 months, sits, crawls and stands by herself.	Walking at 20 months, Cannot walk without help at the time of study.	Walking at 28 months, Running by the time of study, not jumping.	Moderate hypotonia, Walking at 22 months, No further skills.

displays visual impairment diagnosed as cortical blindness. She further has dysmorphic facial features including abnormally shaped ears, periorbital fullness, broad nasal tip, and large nasal root.

There are some unique observations in our study subject. Her MRI showed no occipital lobe atrophy, and she has pachygyria and dilation of lateral ventricles. Instead of low anterior hairline, she has low posterior hairlines. In addition, she has highly arched palate and gingival maldevelopment as well as horizontal nystagmus and left strabismus. Also, she has atrial septal defect.

DOCK7 is detected in all major regions of human brain, including the hippocampus and cortex. *DOCK7* plays a major role in axon development and neuronal polarization.[3] It activates Rac1 and Rac3 Rho small GTPases by exchanging bound GDP for free GTP, and contributes to the STMN1 "Ser-15" phosphorylation during axon formation and neuronal polarization.[4]*DOCK7* controls

neuronal progenitor differentiation by antagonizing TACC3 and regulating interkinetic nuclear migration. This process gives rise to ectopic mitoses and promotes neurons generation rather than self-renewal of progenitors. It is highly probable that the disruption of *DOCK7*'s T-b region induced by the stop-gain mutation (c.C2479T:p.R827X) will devastatingly affect neurogenesis in our study subject. Further, the splice site variation (c.5929-1G>C) is likely to impede the capacity of *DOCK7* in executing axon development and neuronal polarity. Rac1 activated by the *DOCK7* catalytic DHR-2 domain regulates actin and microtubule network dynamics by locally enhancing stathmin/Op18 (Op18) phosphorylation at serine 16, thereby inactivating its actin and microtubule networks, which in turn destabilizes activity of nascent axons.

In conclusion, we identified a pair of novel LOF variants in *DOCK7* to be pathogenic mutations in a Chinese patient affected by EIEE23. Due to the high genetic and phenotypic heterogeneity of EIEE23, some symptoms in our study subject are not quite similar to phenotypes described in previously reported cases. Until now, there is only 1 research paper reporting 3 EIEE23 patients. Our finding broadens the spectrum of *DOCK7* causative mutations and further improves our understanding of the highly heterogeneous clinical symptoms and molecular basis of EIEE23. Our work will aid genetic counseling and gene therapy of EIEE23 in the future.

Declaration of patient consent

The authors certify that they have obtained all appropriate consents for this study. These included the consent by the patient/patient's guardians for their images and other clinical information to be reported in this study. The patient/patient's guardians understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

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Conflicts of interest

None.

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